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(71) Applicant *(for all designated States except US)*: NATIONAL RESEARCH COUNCIL OF CANADA [CA/CA]; 1200 Montreal Road, Ottawa, Ontario K1A 0R6 (CA).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: JAIN, Ravinder, Kumar [CA/CA]; 2413 Irvine Avenue, Saskatoon, Saskatchewan S7J 2A9 (CA). THOMPSON, Roberta, Gail [CA/CA]; 117 Capilano Court, Saskatoon, Saskatchewan S7K 4B9 (CA). ROWLAND, Gordon, Grant [CA/CA]; 213 Lake Crescent, Saskatoon, Saskatchewan S7H 3A1 (CA). McHUGHEN, Alan, Gordon [CA/CA]; 35 Cathedral Bluffs Road, Saskatoon, Saskatchewan S7P 1A1 (CA). MacKENZIE, Samuel, Leonard [CA/CA]; 17 Cambridge Crescent, Saskatoon, Saskatchewan S7H 3P9 (CA). TAYLOR, David, Charles [CA/CA]; 622 Wollaston Bay, Saskatoon, Saskatchewan S7J 4C3 (CA).

(74) Agents: GALE, Edwin, J. et al.; Kirby, Eades, Gale, Baker, Box 3432, Station D, Ottawa, Ontario K1P 6N9 (CA).

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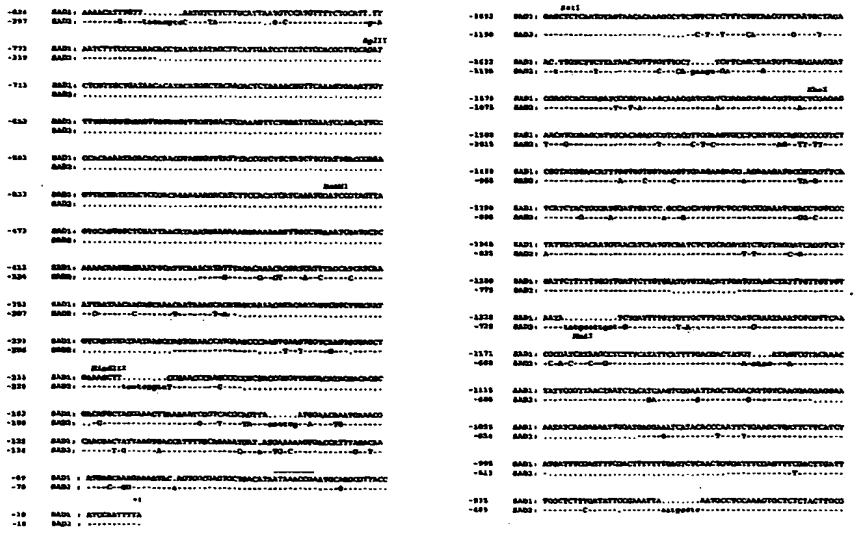

(57) Abstract

The invention relates to promoter sequences obtained from flax and useful for modification of flax and other plants for expression of endogenous or foreign genes. The promoters are the SEQ ID NO. 3 and SEQ ID NO. 4 and are obtained from newly illucidated structures of two SAD genes in flax, namely SEQ ID NO. 1 and SEQ ID NO. 2. The promoters have been inserted into cloning plasmids and deposited at the American Type Culture Collection as plasmids pCDC220 and pCDC214 under deposit numbers ATCC 98192 and ATCC 98193, respectively. The promoters may be used in conjunction with genes to modify characteristics of flax and other plants. The invention includes the SAD genes themselves and DNA sequences substantially homologous to SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, and SEQ ID NO. 4, as well as significant parts thereof.

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FLAX PROMOTERS FOR MANIPULATING GENE EXPRESSION

TECHNICAL FIELD

This invention relates to gene promoters useful for the genetic manipulation of plants. More particularly, the invention relates to gene promoters isolated from flax useful, for example, for manipulating the expression of indigenous genes or transgenes in flax and other plants to modify endogenous characteristics or to introduce new ones.

BACKGROUND ART

Flax (*Linum usitatissimum*) is the second most important oilseed crop in Canada and an important crop worldwide. Unfortunately, the use of flax seed oil is limited by the narrow range of natural fatty acids present in it. Therefore, there is a need to create new cultivars with a wider range of fatty acid composition to supplement the existing food and confections markets (Rowland et al., 1995 - please refer to the "References" section below for full reference identification details). Also, there is a commercial interest in using flax as a vehicle for biofarming of pharmaceutical-related products by molecular genetic manipulation of appropriate transgenes (Moloney and van Rooijen, 1996). A need for flax varieties tolerant to various abiotic and biotic stresses has also been recognized (Rowland et al., 1995). For example, herbicide-tolerant flax varieties would be very useful in crop rotation programs. There is always, of course, a need for promoters useful for expressing foreign genes in various other plants.

Molecular genetic manipulation of flax seed composition or other characteristics, such as stress tolerance, can be achieved by expressing appropriate

transgenes using seed-specific or constitutive gene promoters. While a cDNA sequence corresponding to a flax gene has been reported (Singh et al., 1994), no promoter has yet been characterized from flax. There is, therefore, a need to identify and isolate one or more genes and promoters from flax to facilitate genetic manipulation of the flax plant and other plants.

DISCLOSURE OF INVENTION

An object of the invention is to identify and isolate one or more genes and promoter sequences from flax and to utilize such sequences in the genetic manipulation of plants.

Another object of the invention is to provide a vector containing a promoter sequence from flax for introducing an indigenous gene or a transgene into flax or other plants.

Another object of the invention is to provide a method of modifiying flax and other plants to change characteristics thereof.

Stated in general terms, the present invention is based on the isolation, purification and characterization by the inventors of the present invention of two genes from flax and two promoters from those genes. The sequences obtained are used for regulating the expression of a heterologous gene (foreign, reporter or transgene) in flax and other plant species. This can result in flax plants having different range of fatty acids than natural flax and can result in the development of transgenic plants suitable for the production of specific products or having new and useful characteristics. Such plants and products are of commercial and industrial interest.

According to one aspect of the present invention, there is provided isolated and purified deoxyribonucleic acid of SEQ ID NO:1 or SEQ ID NO:2. These sequences relate to the novel flax genes isolated and characterized by the inventors of the present invention.

These identified and isolated genes are useful in themselves for making antisense or sense constructs based on the derived sequences. Both types of contruct can be used to reduce the levels of similar mRNA during expression of the natural genes. This would result in an increase in 18:0 fatty acid in membrane or storage lipids in flax and other plant species. Sense constructs may also be used in enhancing the levels of mRNA. Such enhancement will result in the increase of 16:1 or 18:1 fatty acids in membranes or storage lipids in flax and other plant species. Such plants will be of increased commercial interest and value.

Thus, according to another aspect of the invention, there is provided a method of changing fatty acids of membrane and storage lipids of plants, characterized by making an antisense or sense construct based on SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4, ligating the constuct into a plant transformation vector, using the vector to transform the genome of a plant or plant seed, and then growing the plant or plant seed and extracting membrane or storage lipids from the plants.

According to another aspect of the invention, there is provided isolated and purified deoxyribonucleic acid of SEQ ID NO:3 or SEQ ID NO:4 (deposited as plasmids ATCC 98193 and 98192, respectively, see details below). These are the promoters that are useful for enhancing or enabling the

expression of genes introduced into flax or other plants.

According to another aspect of the invention, there is provided a gene expression cassette comprising a sequence according to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4. The gene expression cassette is useful in itself as this part of the plasmids mentioned above can be used to construct other plasmid suitable to transform other plant species.

According to yet another aspect of the invention, there is provided a vector for introduction of a gene into a plant cell, the vector comprising a promotor of SEQ ID NO:3 or SEQ ID NO:4.

The invention also relates to transgenic plants and plant seeds having a genome containing an introduced promoter sequence of SEQ ID NO:3 or SEQ ID NO:4 regulating the expression of an introduced gene, and a method of producing such plants and plant seeds.

The invention also relates to substantially homologous DNA sequences (e.g. greater than or equal to 40% homology, more preferably greater than or equal to 70% homology) isolated and/or characterized by known methods using the sequence information of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4, and to parts of reduced length of promoter sequences SEQ ID NO:3 or SEQ ID NO:4 that are still able to function as promoters of gene expression. It will be appreciated by persons skilled in the art that small changes in the identities of nucleotides in a specific promoter sequence may result in reduced or enhanced effectiveness of the promoters and that partial promoter sequences often work as effectively as the full length versions. The ways in which promoter sequences can be varied or shortened are well known to

persons skilled in the art, as are ways of testing the effectiveness of promoters. All such variations of the promoters are therefore claimed as part of the present invention.

It should be noted that the term "promoter" in this disclosure includes the core promoter elements (TATA box and initiator) and upstream regulatory elements (enhancers)(Datla et al., 1997).

As will be appreciated from the description above, the promoters of the invention are beneficial in manipulating the expression of genes in flax and other crops.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows genomic DNA sequence of the SAD1 [SEQ ID NO:1; identified in Fig. 1 as LUSAD1.SEQ] and SAD2 [SEQ ID NO:2; identified in Fig. 1 as LUSAD2.SEQ] genes and the corresponding SAD cDNA sequence [SEQ ID NO:5; identified in Fig. 1 as LUCDNA]. Nucleotides (nt) are represented by capital letters. Nucleotides different from the cDNA sequence are shaded, including those of introns. Differences between SAD1 and SAD2 are shown in shaded lower case letters. Gaps in the sequences are presented by dashes. The start and stop codons on the cDNA sequence are boxed.

Fig. 2A is a partial restriction map of the SAD1 gene, and Fig. 2B shows the result of a DNA blot analysis identifying the regulatory sequences of SAD1 and SAD2.

Fig. 3 shows an outline of the scheme employed to isolate the promoter regions of the two SAD genes. Position and direction of the primers used in IPCR are indicated by arrowheads. Various abbreviations are as follows: E, exon; I, Intron; RE, 5'- regulatory elements (promoters); and UT, untranslated regions.

Fig. 4 discloses nucleotide sequences [SEQ ID NO:3 (SAD1) and SEQ ID NO:4 (SAD2)] of the 5'- regulatory regions of the two SAD genes. Homologous nt are represented by a dash (-), gaps by a dot (.), and additions by lower case letters. A putative transcriptional site is indicated by +1, and a TATA box is overlined. Key restriction sites are also shown.

Fig. 5 shows salient features of the plasmids CDC214 and pCDC220. Various abbreviations are as follows: flax promoter I, SAD1 gene promoter; flax promoter II, SAD2 gene promoter; *GUS* (*uidA*), gene for β-glucuronidase enzyme; *nos*-T, transcriptional terminator of the nopaline synthase gene; *nptII*, neomycin phospho-transferase expression cassette. The arrowheads indicate the direction of transcription. Key restriction sites are shown. Regions outside the left and right border (LB and RB) are that of a previously described binary plant transformation vector, pRD410 (Datla et al., 1992).

Fig. 6 shows the expression of a heterologous gene (*uidA*) by the two SAD gene promoters in various tissues of flax. Different tissues are abbreviated as YL+A, young leaves and apices; ML, mature leaves: S, stems; R, roots; B, buds; 1/2 OF, half open flower; Fl, Flower; and MS, seeds at about mid-development. Data presented are from one generation of two plants transformed with a tandem 35s promoter (2x35s), two generations of two plants transformed with pCDC214 (SAD1), and one generation of two plants transformed with pCDC220(SAD2).

Fig. 7 shows the expression of a heterologous gene(*uidA*) by the two SAD gene promoters during flax seed development and in relation to fatty acid and protein biosyntheses. For GUS assays, data represent

one generation of two plants transformed with a tandem 35s promoter (2x35s), two generations of two plants transformed with pCDC214(SAD1), and two generations of a plant transformed with pCDC220 (SAD2). For fatty acids, three individual embryos of var. McGregor were analyzed. For protein content, data are from two transgenic plants transformed with pCDC214 and 220.

Fig. 8 shows the expression of a heterologous gene(*uidA*) by the two SAD gene promoters in tobacco leaves and mid-developmental seeds. Data represent 5 to 8 transgenic plants transformed with pCDC214 (SAD1), pCDC220 (SAD2), pRD410 (35s), and pRD420 (*uidA* alone).

Fig. 9 shows the expression of a heterologous gene(*uidA*) by the two SAD gene promoters during tobacco seed development. Various developmental stages of tobacco seeds were identified according to de Silva et al. (1992) and are abbreviated as W, white; LB, light brown; B, brown; DB, dark brown; and M, mature. Data represent 5 to 8 transgenic plants transformed with pCDC214 (SAD1), pCDC220 (SAD2), pRD410 (35s), and pRD420 (*uidA* alone).

Fig. 10 shows the expression of a heterologous gene(*uidA*) by the two SAD gene promoters in canola leaves and mature seeds. Data represent 2 to 5 plants transformed with pCDC214 (SAD1), pCDC220 (SAD2), pRD410 (35s), and untransformed plants (UT).

BEST MODES FOR CARRYING OUT THE INVENTION

In flax, endogenous SAD activity can be detected from about 10 days after pollination (dap) to seed maturity, suggesting a promoter of this gene would be useful in manipulating gene expression during seed development. Moreover, SAD has been found to be the key enzyme in manipulating the levels of saturated fatty acids in rapeseed and soybean triacylglycerols

(Knutzon et al., 1992; see Töpfer et al., 1995). During studies carried out by the inventors aimed at diversifying flax as a crop, it was discovered that there are two SAD genes in flax. The isolation, purification and characterization of these genes and their promoters is disclosed below, as well as the expression capabilities of the promoters in flax and other plant species.

The promoters developed according to the present invention can be used to modify an endogenous characteristic of flax or another plant species, or to to add a new characteristic. An example of a modification of an endogenous characteristic of flax is, for example, the alteration of levels of different types of fatty acids in the seed oils. The introduction of a new characteristic is, for example, the production of a thermoplastic polymer in plants that normally do not produce thermoplastics. While it is normally easy to detect added characteristics, it is sometimes difficult to detect altered characteristics because of natural variation of characteristics in plants. The alterations can, however, be detected by comparing the average characteristics of a statistically significant number of the plants under examination with a statistically significant number of genomically-unmodified plants of the same genotype, grown under identical environmental conditions at the same time. If there is an appreciable difference in the measured characteristic, then it can be said that there has been an alteration of that characteristic and that the alteration is a result of the genomic-modification.

In the case of an added characteristic, again the comparison can be made with genomically-unmodified

plants of the same genotype, again grown under identical environmental conditions at the same time.

The promoters of the present invention belong to a two-member gene family encoding the enzyme Δ9 desaturase (Stearoyl-acyl carrier protein desaturase; SAD; EC 1.14.99.6). Stearoyl-acyl carrier protein desaturase is the first enzyme in the fatty acid desaturation pathway, and it catalyzes the conversion of stearoyl-ACP(18:0-ACP) to oleoyl-ACP(18:1Δ9-ACP). The promoters were isolated using the inverse polymerase chain reaction (IPCR) technique. They are capable of expressing a foreign gene, e.g. *uidA* (which encodes β-glucuronidase: GUS), in various tissues with high level of expression in seeds.

In developing seeds, both promoters showed a similar temporal expression pattern for *uidA* (measured as GUS activity). The GUS activity could be detected as early as 4 dap in developing seeds and in desiccated seeds (~50 dap) of transgenic flax. In developing seeds, the ability of the promoters to effect *uidA* gene expression correlated well with both fatty acid and protein biosyntheses and the maximum activity of GUS preceded the maximal accumulation of fatty acids and proteins.

The promoters of the invention are useful in manipulating transgene expression in a variety of tissues including seeds. Some of the products which are possible using these promoters include, but are not limited to, the following: plants with enhanced herbicide, pest, pathogen, and stress resistance; plants containing oil, protein, and carbohydrate of altered composition and content; plants with reduced anti-nutritional substances; plants producing

pharmaceutical compounds such as antibodies, neuropeptides, recombinant proteins, and biodegradable thermoplastics (Bennett, 1993; Moloney and van Rooijen, 1996; Datla et al., 1997).

The effectiveness of the promoters of the present invention is predictable from the effectiveness of known promoters. For example, it is well established that promoters such as cauliflower mosaic virus (CaMV) are capable of expressing a wide variety of genes in a wide varity of plant species. Napin promoter (from rapeseed) has been used to express a variety of genes in canola/rapeseed (Knutzon et al., 1992; Jones et al., 1995; Dahesh et al., 1996). Phaseolin gene promoter (from bean) has also been used to express several genes in rapeseed (Hitz et al., 1995). The β-conglycinin promoter (from soyabean) has been used to express genes not only in soyabean but also in Petunia (Kinney, 1997; Chen et al., 1986).

Moreover, by testing the promoters in two very diverse plant species, as will become apparent from the experimental detail below, the inventors have demonstrated that the promoters would function in other diverse plant species as well.

Further demonstration of this principle can be obtained from Chen ZL, Schuler MA, Beachy RN. 1986; Dehesh K, Jones A, Knutzon DS, Voelker TA. 1996; Hitz WD, Mauvis CJ, Ripp KG, Reiter RJ, DeBonte L, Chen, Z. 1995; Jones A, Davies HM, Voelker TA. 1995; Kinney, AJ. 1997; and Knutzon et al., 1992.

It is believed that the present invention can now best be described by presenting experimental details forming a specific illustration. It should be kept in mind, however, that the present invention is not limited to these details.

EXPERIMENTAL DETAILS

Molecular Biological Techniques

Isolation of plasmid DNA, restriction digestion, modification and ligation of DNA, PCR, gel electrophoresis, and transformation and culture of *E. coli* strains were carried out according to standard procedures (Sambrook et al., 1989). Nucleotide sequencing was performed using double stranded plasmid DNA by the dideoxy chain termination method (Sanger et al., 1977) using a *Taq* DYEDEOXY™ terminator cycle sequencing kit (available from Applied Biosystems) and an Applied Biosystems Model 370A Sequencer (available from Applied Biosystems). The oligodeoxy-ribonucleotides used in nucleotide sequencing, and PCR techniques were synthesised using a phosphoramidate synthesis procedure in a Biosearch 8750 DNA synthesizer (New Brunswick Scientific Co.), and purified by HPLC-based protocols (Gait, 1984). IPCR was done according to Ochman et al. (1993) and Warner et al. (1993).

Plant DNA was extracted using the protocol of Dellaporta et al. (Dellaporta et al., 1983) except that RNA was removed by adding 100 µg of RNAase B (Sigma) followed by incubation at 65°C for 20 min. The DNA was extracted once with an equal volume of phenol:chloroform (1:1, v/v) and once with an equal volume of chloroform:isoamyl alcohol (24:1, v/v). Five µg of DNA was digested with the appropriate restriction enzyme, fractionated on a 0.8% agarose gel, and pressure-blotted onto Hybond-N™ nylon membranes (Amersham) using the PosiBlot™ apparatus (Stratagene) after depurination, denaturation and neutralization of the DNA (Sambrook et al., 1989). The blotting solution contained 0.02 M NaOH and 1 M NH_4-acetate. The DNA was

immobilized on the membrane by baking the membranes at 80°C for 1 h.

A radioactive probe for identifying promoters was prepared by annealing 10 ng of oligo-29A and 30A (Table 1 below) and then filling in the ends using the Klenow fragment of DNA polymerase and random primer kit solutions (GIBCO BRL).

Table 1

Nucleotide sequence of various oligonucleotides (OL) used

OL-24 (-)	5'-GAA_{1371}ATGCCATCAT-ACTCCAATCAT-3' [SEQ ID NO:6]
OL-25 (+)	5'-GAA_{120}CCTTCAACAAC-AATGGCTCTC-3' [SEQ ID NO:7]
OL-29A (+)	5'-$_{120}$CCTTCAACAACAATGGCTCTCAAGC-TCAACCCAGTCACCACCTT-3' [SEQ ID NO:8]
OL-30A (-)	5'-$_{194}$GGAGAAGTTGTTGAGGGAGCGTGTT-GAAGGGAAGGTGGTGACTGGGTTGA-3' [SEQ ID NO:9]
OL-39 (-)	5'-$_{253}$TTGGTGGAGGTGGAACTGAA-3' [SEQ ID NO:10]
OL-110 (+)	5'-$_{263}$AGCTAAAGAAGTCACATGGAC-3' [SEQ ID NO:11]

NOTE: The number in subscript corresponds to the nucleotide residue in the SAD cDNA sequence (Singh et al., 1994). + and - indicate coding and non-coding strand.

The sequence of oligo-29A corresponded to nt 120-163 of SAD cDNA (reported by Singh et al., 1994). The sequence of oligo-30A corresponded to nt 145-194. In this way, radioactive probe fragments spanning 75 bps in the 5' end region of SAD cDNA were obtained.

Prehybridization was done at 65°C for 3 h in 5x SSPE, 5x Denhardts solution, 0.5% SDS, and 500 µg of

Salmon sperm DNA (Amersham). Hybridization was done at 55°C for 18 h. The membrane was washed at room temperature in 2x SSPE and 0.1% SDS for 15 and 5 min and then at 50°C in 1x SSPE and 0.1% SDS for 10 min. At this point the membrane was free of background signal. Autoradiograms were obtained by exposing the membranes for variable lengths of time to Kodak X-OMAT™ AR films with intensifying screens at -70°C.

Reporter Gene Constructs

A 1.747 kb DNA fragment containing only the 5'-regulatory region and a part of the untranslated region of the SAD1 gene was amplified by PCR and cloned into the pCRII vector (Invitrogen Corp). The same fragment was retrieved as an *Eco*RI fragment from the pCRII vector and subsequently cloned into pBluescript™ II SK (Stratagene) to gain some cloning sites. The relevant 5'- regulatory region, approximately 1.257 kb, of the SAD2 gene was PCR-amplified but using the *pfu* DNA polymerase (Stratagene), and cloned into an *Eco*RV site of the pBluescript II SK vector.

The SAD1 and SAD2 gene 5' regulatory elements were cloned into pRD420 as a *Sal*I-*Sma*I fragment in front of the *uidA*. The plasmid pRD420 was obtained from Dr. R.S.S. Datla, NRC Plant Biotechnology Institute, 110 Gymnasium Place, Saskatoon, Saskatchewan, Canada, S7N OW9 (Datla et al., 1992). The resulting constructs were labeled as pCDC214 and pCDC220. These constructs were deposited on October 3, 1996 (tested for viability on October 9, 1996, deposit receipt dated October 10, 1996) under the terms of the Budapest Treaty at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852, USA, under deposit nos. ATCC 98193 and 98192, respectively. The plasmids CDC214 and 220

were transferred directly to *Agrobacterium* strain GV3101 containing helper plasmid pMP90 (Koncz and Schell, 1986) using a freeze-thaw method of transformation (An et al., 1988).

Plant Transformation

Flax seeds were surface sterilized by stirring in 70% ethanol for 2 minutes, followed by three 10 minute washes in 0.5% sodium hypochlorite (freshly diluted from the commercial product), and 5 rinses in sterile distilled water. Seeds were germinated on basal medium consisting of Murashige and Skoog (MS) major and minor salts and Gamborg vitamins (Sigma 0404), 3% sucrose and 0.8% agar. The pH of the medium was adjusted to 5.8 before autoclaving. About 10 surface-sterilized seeds were placed in each 100x15 mm plate. The plates were sealed with parafilm and placed in the dark at 22°C for 5 to 7 days.

Derivatives of *Agrobacteria tumefaciens* strain GV3101/pMP90 carrying pCDC214 and pCDC220 were grown on solidified 2x YT medium (Sambrook et al. 1989) supplemented with 50 µg/ml kanamycin and 50 µg/ml gentamycin sulfate. Single colonies from 2 to 3 day-old culture plates were used to inoculate 10 ml liquid 2x YT medium containing antibiotics as above and 20 µM acetosyringone. Cultures were grown at 28°C with rotary agitation for about 24 hours. Prior to inoculation of flax tissues, the cell concentration of the suspension was adjusted to $1x10^9$ cells/ml.

The following methods for obtaining transformed flax callus were modified from Mlynárová et al. (1994). Hypocotyls of 5-7 day aseptic flax seedlings were cut into segments 3-4 mm long. To avoid dehydration, the segments were maintained in a small volume of liquid

basal medium until all the hypocotyls were cut. The hypocotyl segments were immersed in bacterial suspension ($1x10^9$ cells/ml) for 30 minutes with occasional swirling. The suspension was removed by aspiration and the hypocotyl segments were transferred to sterile filter paper to remove excess liquid. The segments were placed on agar-solidified (0.8%) basal medium supplemented with 4.44 μM 6-benzylaminopurine and 0.54 μM naphthaleneacetic acid (MSD4x2 medium; Basiran et al., 1987). Maltose (3%) replaced sucrose as the carbohydrate source. About 25 explants were placed in each 100x15 mm Petri dish and maintained at 22-24°C, with a 16 h photoperiod and photon density of approximately 50 $\mu mol/m^2/s$. After 2 days the segments were transferred to the same medium supplemented with 100 μg/ml kanamycin for selection of transformed cells and 200 μg/ml cefotaxime to eliminate *Agrobacteria*. The explants were maintained under the same growth conditions for 3 weeks. As a control, non-inoculated segments were treated in the same way.

Green callus formed at the cut ends of most of the inoculated hypocotyl segments, whereas little or no callus appeared on non-inoculated segments and they were completely bleached after 3 weeks on the selection medium. Callus was excised and transferred to basal medium (3% maltose) supplemented with 5 μM zeatin and antibiotics as above. Shoots regenerated from some of the calli within 3-4 weeks.

When the shoots had elongated to 0.5 to 1.0 cm, they were removed from the callus and placed in capped glass tubes (100x25 mm) containing 8 ml rooting medium: 1/2 strength MS salts, 3% sucrose, 0.1 μM IAA, 0.8% agar, pH 5.8, and 30 μg/ml kanamycin for selection of

transformed shoots. The shoots were maintained under low light (<25 $\mu mol/m^2/s$) for 6-8 days by which time some of the shoots had roots about 2-3 mm long. The plantlets were transferred to pots in the growth chamber within 10-14 days, when roots had elongated to about 2 cm and the shoots were 3-5 cm tall. Transgenic plants were grown under 18 h of light (300-500 $\mu mol/m^2/s$) and day/night temperature of 20/17°C. The plants were fertilized just before flowering with a solution containing 27 g of 15N:30P:15K supplemented with 0.9 g $CuSO_4$ in 9 liters of water.

Transformation of canola and tobacco were performed according to Moloney et al. (1989) and Horsch et al. (1985), respectively.

Tissue Sampling

Various tissues and developing seeds at different stage of development were harvested and immediately frozen in liquid N_2 and stored at -80°C until analyzed.

In progeny generations, these tissues were combined from a total of 8 plants.

Fluorimetric GUS Enzyme Assay

Fluorimetric GUS assay was done essentially according to Jefferson (1987). The assays were done in a micro well titer plate and fluorescence of the reactions was measured by CytoFluor™ II multi-well fluorescence plate reader (PerSeptive Biosystems).

Determination of Fatty Acid and Protein Content in Seeds

The fatty acid content of seeds of different ages was determined by fatty acid methyl ester analysis of seed homogenates as described previously (Taylor et al., 1992).

The same protein extracts which were used for GUS

assays were used for protein estimation. Protein concentration was determined using a modified Bradford assay method (Bio-Rad protein assay) and BSA as the standard.

RESULTS

Isolation and characterization of the two SAD genes

The inventors of the present invention have found that three lines of evidence prove there are two SAD genes in flax, namely: the amplification of two different sized DNA fragments by PCR, the results of restriction analysis of cloned PCR products, and the results of DNA blot analysis of flax genomic DNA.

The genomic sequences of the two SAD genes were amplified by PCR. Several oligonucleotide primers were synthesized based on the nucleotide sequence of the published SAD cDNA sequence (Singh et al., 1994). These primers were used in all possible combinations with flax genomic DNA as the template to amplify different segments of SAD genes. The molecular size of the PCR products was determined by agarose gel electrophoresis; in most reactions two products of very similar molecular size were detected, suggesting the possibility of two SAD genes in flax. Amplification with oligo-25 and 24 (Table 1) yielded a fragment of about 2.6 kb. This fragment contained the whole SAD gene as determined by sequence data.

The amplified SAD gene fragments were cloned into pCRII vector (Invitrogen Corp.). The identity of the amplified gene products was confirmed by comparison of their nucleotide sequences with the SAD cDNA sequence (Singh et al., 1994). Sequence analyses indicated that the SAD1 and SAD2 genes have 97.2% similarity with each other in the coding region and 96.2% and 93.7% with the published flax cDNA sequence, respectively (Fig. 1).

It is clear that the mRNA for SAD cDNA, reported by Singh et al. (1994), was transcribed from the SAD1 gene. Some general features of the flax SAD genes have been deduced from sequence analysis. As expected on the basis of the cDNA sequence, the coding region of the gene is 1191 bps. This consists of three exons interrupted by two introns of approximately 0.6 to 0.7 kb. Exon 1 consists of 123 bp, whereas exons 2 and 3 are 507 bp and 561 bp long, respectively.

Verification for the presence of two SAD genes in flax comes from the analyses of two independent clones, each containing the full length gene. Although the nucleotide sequences of the coding regions are almost identical, there are several base changes. One of these has altered a restriction enzyme site, *Nco*I, resulting in the observation that the two clones have different restriction digestion patterns. The two clones also differ significantly in their intron sequences (Fig. 1). The different intron sequences are presumably responsible for the slight difference in the molecular size of the two PCR products generated by the same primer combination.

Identification of SAD Gene Promoter Sequences in Flax Genome

Genomic DNA was extracted from 7-10 days old seedlings of flax var. McGregor (obtained from Dr. G. Rowland, Crop Development Centre, 51 Campus Dr., Saskatoon, Saskatchewan S7N 5A8), digested with restriction enzyme, *Bam*HI, *Bcl*I, *Bgl*II, *Nde*I or *Sst*I, gel-fractionated and blotted onto nylon membrane for probing. These restriction enzymes would cut within the flax SAD genomic sequence as indicated in Figure 2A and elsewhere in the flax genome. When the DNA blot was hybridized with the probe, DNA fragments containing

the 5'- upstream region and a part of the 5'- untranslated and coding region of the SAD gene were expected to hybridize (Figure 2A).

The result of one such experiment is shown in Figure 2B. In each lane, two different size fragments hybridized with the probe indicating the existence of two SAD genes in flax. Singh et al. (1994) have shown only one SAD gene in flax. Since both the genes might be active, the inventors decided to isolate the 5' regulatory DNA sequences of both SAD genes.

Isolation and Characterization of Promoter Elements

5'- regulatory DNA sequences of the two SAD genes were amplified using the IPCR technique.

DNA blot analysis of the flax genome indicated that the two fragments obtained from the digestion of flax DNA with the restriction enzyme *SstI* would contain about 1.7 and 1.2 kb of 5' flanking regions of the SAD1 and SAD2 gene, respectively (Figs. 2B, 3 and 4). These fragments are expected to contain sufficient 5'- regulatory elements required for gene expression. *SstI* was used to cut the flax genomic DNA, and the circularized DNA template required for IPCR was prepared. An outline of the promoter isolation scheme is shown in Fig. 3, and is believed to be self-explanatory.

Flax genomic DNA was digested with the restriction enzyme *SstI* and gel fractionated. DNA fragments were isolated from a region of the agarose gel where the two promoter fragments that hybridized with the SAD probe were expected (Fig. 2B and 3). These DNA fragments were ligated at a concentration favoring the circularization of single DNA molecules (Ochman et al., 1993; Warner et al., 1993). The circularized DNA was then used as a template in the IPCR with two primers

(oligo-39 and oligo 110; Table 1). The orientation of the each member of the primer set used in the IPCR is opposite to that normally used in a regular PCR (Fig. 3). Two distinct fragments of the expected sizes, 2.2 kb and 1.7 kb, were amplified using IPCR. The untranslated region and parts of the exon 1 and exon 2 constituted the additional approximately 0.5 kb (Fig.3). The two fragments could also be digested with *Sst*I indicating the authenticity of the PCR product.

The two DNA fragments were cloned in the pCRII vector (Invitrogen Corp.) and sequenced. The DNA sequence of the 5'- regulatory regions of the two SAD genes was compiled and compared (Fig. 4). The two SAD promoters are quite homologous. A large deletion of 368 bp in the SAD2 gene promoter (corresponding to nt 759 to 391 in the SAD1 promoter) is very conspicuous. There are a few short deletions, some substitutions and minor gaps in both the promoters. Based on the sequence data, 3'- regions of these DNA fragments were matched with the 5'- coding regions of the two SAD genes, and thereby assigned the promoters to their respective SAD genes.

Expression of the β-glucuronidase Gene by Flax Promoters in Transgenic Plants

The ability of a promoter to regulate expression of a gene spatially and temporally can be demonstrated by using it to express a heterologus gene. To achieve this here, first, reporter gene constructs were made by fusing the promoter of the SAD1 or SAD2 gene with the *uidA* gene (Fig. 5). These expression constructs were then used to transform flax, canola and tobacco, and independent transgenic plants of these species were obtained.

Different tissues were sampled and assayed for GUS activity to determine spatial or tissue-specific expression. Developing seeds were also collected at various stages of development to analyse the temporal expression pattern of the two promoters during seed development.

These promoters were capable of expressing the *uidA* gene in various tissues, with high level of expression in seeds (Fig. 6). In developing seeds, both the promoters showed similar temporal expression patterns for GUS (Fig. 7). The GUS activity could be detected as early as 4 dap in developing seeds and in desiccated seeds (approximately 50 dap) of transgenic flax with higher activities around mid-development (14 to 28 dap).

In tobacco, GUS activity in leaf was insignificant with both the promoters whereas in seeds GUS activity could be detected easily (Fig. 8). In developing tobacco seeds, GUS activity was highest at about mid-development (Fig. 9). In canola, GUS activity could be detected easily in both leaves and seeds (Fig. 10).

Utility of the Flax Promoters in Regulating Gene Expression

The utility of the flax promoters disclosed here is demonstrated by comparing their effect on *uidA* gene expression with both lipid and protein biosynthesis in developing flax seeds. In developing seeds, *uidA* expression correlated well with both fatty acid and protein biosynthesis (Fig. 7). In seeds, maximum expression of the *uidA* gene controlled by the SAD gene promoters preceded the maximum accumulation of fatty acids and proteins. Also, in tobacco the temporal pattern of *uidA* gene expression correlated well with the lipid biosynthesis (de Silva et al., 1992).

Therefore, these promoters are useful in manipulating gene expression in seeds. Since these promoters are also active in other tissues they are useful in manipulating gene expression in a variety of tissues.

Utility of SAD Genes

The utility of the genes can be demonstrated by carrying out the following predictive experiments (similar experiments have been reported in Knutzen et al., 1992; Topfer et al., 1995). Firstly, antisense or sense constructs are made using the disclosed or other promoters. For example, these genes or their parts can be ligated into a SmaI restriction site of pCDC 214 or 220 (Fig. 5) or any other convenient cloning site of another plant transformation vector. These recombinant plasmids can then be mobilized, for example, into an *Agrobacterium* strain which can then be used to transform a variety of plant species. Any changes in fatty acids of membrane and storage lipids can be evaluated by routine methods described in this application.

Both type of constructs are expected to reduce the levels of similar mRNA during expression of the natural genes resulting in an increase of 18:0 fatty acid in membrane or storage lipids. Sense constructs can also be used in enhancing the levels of mRNA. Such enhancement will likely result in the increase of 16:1 or 18:1 fatty acids in membranes or storage lipids of plants. Such plants will be of increased commercial interest and value.

It will be appreciated by persons skilled in the art that various modifications and alterations may be made to the present invention without departing from the general scope of the invention as defined by the

following claims. All such variations and modifications should be considered part of this invention.

5

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Ravinder Kumar Jain
(B) STREET: 2413 Irvine Avenue
(C) CITY: Saskatoon
(D) STATE: Saskatchewan
(E) COUNTRY: Canada
(F) POSTAL CODE (ZIP): S7J 2A9

(A) NAME: Roberta Gail Thompson
(B) STREET: 117 Capilano Court
(C) CITY: Saskatoon
(D) STATE: Saskatchewan
(E) COUNTRY: Canada
(F) POSTAL CODE (ZIP): S7K 4B9

(A) NAME: David Charles Taylor
(B) STREET: 622 Wollaston Bay
(C) CITY: Saskatoon
(D) STATE: Saskatchewan
(E) COUNTRY: Canada
(F) POSTAL CODE (ZIP): S7J 4C3

(A) NAME: Gordon Grant Rowland
(B) STREET: 213 Lake Crescent
(C) CITY: Saskatoon
(D) STATE: Saskatchewan
(E) COUNTRY: Canada
(F) POSTAL CODE (ZIP): S7H 3A1

(A) NAME: Alan Gordon McHughen
(B) STREET: 35 Cathedral Bluffs Road
(C) CITY: Saskatoon
(D) STATE: Saskatchewan
(E) COUNTRY: Canada
(F) POSTAL CODE (ZIP): S7P 1A1

(A) NAME: Samuel Leonard MacKenzie
(B) STREET: 17 Cambridge Crescent
(C) CITY: Saskatoon
(D) STATE: Saskatchewan
(E) COUNTRY: Canada
(F) POSTAL CODE (ZIP): S7H 3P9

(ii) TITLE OF INVENTION: Flax Promoters For Manipulating Gene Expression

(iii) NUMBER OF SEQUENCES: 11

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 60/029,416
(B) FILING DATE: 30-OCT-1996

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2701 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Linum usitatissimum
(B) STRAIN: McGregor

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

ACAACCATTC AATTCAAAAG TTTTTCCAAT TTCCATTTCC TCATCTGCCT TACCCATAAA 60

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T 2701

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2705 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Linum usitatissimum
(B) STRAIN: McGregor

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1693 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Linum usitatissimum
(B) STRAIN: McGregor

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

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CAAGTCGGAA TTAGCTAGAC ATTGTCAAGG AGGAGGAAAA TATCAAGAAA ATTGGATGAG 660

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(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1191 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Linum usitatissimum
(B) STRAIN: McGregor

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GAGCTCTCAA TGTAGTAACA CAAACTCTTT TTTTTCCATA ACGTTGAATG TTAGAACTTT 60

GTCTTTTTAT AACTGTTTCT TTCATGAAGC TGATCAGCTG ATGTTGGAGA AGGATGGAGC 120

CACGGAGATT CCTGAAAAGC AAAGGATGGA ACGAGAGGAG ACGGTGACTC GAGAGTACAG 180

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(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1371 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Linum usitatissimum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CGACAACCAT TCAATTCAAA AGTTTTTCCA ATTTCCATTT CCTCATCTGC CTTACCCATA 60

AATCTCGACG GACACCAAAA AACTCAGCCA GCTTGCCCCC AAACAACAGC GCAGAAAAAC 120

CTTCAACAAC AATGGCTCTC AAGCTCAACC CAGTCACCAC CTTCCCTTCA ACACGCTCCC 180

TCAACAACTT CTCCTCCAGA TCTCCTCGCA CCTTTCTCAT GGCTGCTTCC ACTTTCAGTT 240

CCACCTCCAC CAAGGAGGCT GAAGCTAAAG AAGTCACATG GACCACCAAA AGAGGTGCAT 300

ATGCAAGTGA CCCATTCCAT GCCCCCACAG GAAGCTGGGA GATATTTAAG TCTCTGGGAA 360

GGTTGGGGCT GAGGGATGTT CTTATTTCGC ACCTGAAGCC AGTTGAGAAA TGCTGGCAGC 420

CACAGGATTT CCTGCCCGAA CCTGAGTCGG ATGGGTTCGA GGAGCAAGTG AAGGAGCTCA 480

GGGCAAGGGC CAAAGAACTG CCCGATGACT ATTTTGTTGT GCTGGTTGGG GATATGATCA 540

CCGAAGAAGC TCTGCCGACT TACCAGACAA TGCTCAACAC CCTTGACGGG GTGAGGGACG 600

AGACTGGAGC CAGCCTTACG CCGTGGGCAA TCTGGACAAG GGCGTGGACC GCTGAAGAGA 660

ATAGGCACGG TGACCTTCTC AACAAGTATC TATACCTCTC TGGAAGGGTG GACATGAGGC 720

AAATTGAAAA GACCATTCAG TATCTCATCG GCTCTGGAAT GGATCCAAAA ACAGAAAACA 780

ACCCCTACCT CGGTTTCATC TACACCTCAT TCCAAGAGAG GGCAACGTTC ATCTCCCACG 840

GAAACACAGC CAGACTCGCC AAGGACCATG GGGACATGAA GCTGGCGCAG ATCTGCGGGA 900

TCATCGCAGC AGACGAGAAA CGGCACGAAA CCGCATACAC CAAGATCGTC GAGAAGCTCT 960

TCGAGATCGA CCCTGACGGT ACAGTGCTGG CACTGGCGGA CATGATGAGG AAGAAGATAT 1020

CGATGCCCGC CCACTTGATG TACGATGGAG AAGACGACAA CCTCTTCGAC AATTACTCGT 1080

CAGTCGCTCA ACGCATCGGG GATACTGCCA AGGATTATGC CGATATCCTG GAGTTCCTGG 1140

TGGGGAGGTG GAAAGTGGAT GCTTTTACGG GGCTTTCCGG GGAAGGGAAC AAAGCTCAGG 1200

ATTTTGTCTG CGGGCTTCCT GCGAGGATTC GAAAGTTGGA GGAGAGGGCT GCGGGGAGGG 1260

CAAAGCAAAC GTCGAAATCT GTCCCGTTCA GCTGGATCTT CAGCAGAGAA TTGGTACTCT 1320

AATGGAGTTT GCTTGAGAGT AGAGTGTGGA ATGATTGGAG TATGATGGCA T 1371

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Linum usitatissimum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GAAATGCCAT CATACTCCAA TCAT 24

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Linum usitatissimum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GAACCTTCAA CAACAATGGC TCTC 24

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Linum usitatissimum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

CCTTCAACAA CAATGGCTCT CAAGCTCAAC CCAGTCACCA CCTT 44

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Linum usitatissimum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GGAGAAGTTG TTGAGGGAGC GTGTTGAAGG GAAGGTGGTG ACTGGGTTGA 50

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Linum usitatissimum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

TTGGTGGAGG TGGAACTGAA 20

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Linum usitatissimum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

AGCTAAAGAA GTCACATGGA C 21

REFERENCES OF INTEREST TO THE PRESENT INVENTION

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CLAIMS:

1. Isolated and purified deoxyribonucleic acid (DNA), characterized in that said DNA includes a sequence according to SEQ ID NO:1 or SEQ ID NO:2, or a sequence that is substantially homologous thereto.

2. Isolated and purified deoxyribonucleic acid (DNA), characterized in that said DNA includes a sequence according to SEQ ID NO:3 or SEQ ID NO:4, or a sequence that is substantially homologous thereto or that is a part of SEQ ID NO:3 or SEQ ID NO:4.

3. A vector for introducing at least one gene into plant cells, characterized in that said vector contains a promoter having a sequence according to SEQ ID NO:3 or SEQ ID NO:4, or a sequence that is substantially homologous thereto, or that is a part of SEQ ID NO:3 or SEQ ID NO:4.

4. A vector according to claim 3, characterized by further including at least one gene under expression control of said promoter.

5. Plasmid pCDC220 (ATCC 98192).

6. Plasmid pCDC214 (ATCC 98193).

7. An isolated and purified gene expression cassette characterized by containing a sequences selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4, or a sequence that is substantially homologous thereto, or that is a part of said sequence.

8. A plant characterized by having a genome containing an introduced nucleotide sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4, or a sequence that is substantially homologous thereto, or that is a part of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4.

9. A plant seed characterized by having a genome containing an introduced nucleotide sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4, or a sequence that is substantially homologous thereto, or that is a part of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4.

10. A plant as claimed in 8, characterized by exhibiting an alteration of an endogenous characteristic or an addition of a new characteristic compared to a genomically-unmodified plant of the same genotype.

11. A plant seed as claimed in 9, characterized by exhibiting an alteration of an endogenous characteristic or an addition of a new characteristic compared to a genomically-unmodified plant seed of the same genotype.

12. A method of producing transgenic plant by introducing a gene into a genome of said plant under control of a promoter, characterized in that said promoter is of SEQ ID NO:3 or SEQ ID NO:4, or a sequence that is substantially homologous thereto, or that is a part of SEQ ID NO:3 or SEQ ID NO:4 and that the said gene could of SEQ ID NO:1, SEQ ID NO:2, or a

sequence that is substantially homologous thereto, or that is a part of SEQ ID NO:1, SEQ ID NO:2.

13. A method according to claim 12, characterized in that said plant is flax.

14. A method of producing a transgenic plant seed by introducing a gene into a genome of a plant under control of a promoter to produce a transgenic plant, growing said plant and obtaining transgenic seeds therefrom, characterized in that said promoter is of SEQ ID NO:3 or SEQ ID NO:4, or is a sequence that is substantially homologous thereto, or that is a part of SEQ ID NO:3 or SEQ ID NO:4, and that the said gene could of SEQ ID NO:1, SEQ ID NO:2, or a sequence that is substantially homologous thereto, or that is a part of SEQ ID NO:1, SEQ ID NO:2.

15. A method of claim 14, characterized in that said plant is selected from flax, canola and tobacco.

16. A DNA sequence characterized in that the sequence is substantially homologous to at least a part of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4, and in that said sequence has been isolated or characterized using sequence information from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4.

17. A method of changing fatty acids of membrane and storage lipids of plants, characterized by introducing an antisense or sense construct based on SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4 into a plant transformation vector, using the vector to transform the genome of a plant or plant seed, and then

growing the plant or plant seed and extracting membrane or storage lipids from the plants or plant seeds.

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LUCDNA.SEQ  CGACAACCATTCAATTCAA.....AAGTTTTTCCAATTTCCATTTCCTCATCT  60
LUSAD1.SEQ  ..ACAACCATTCAATTCAA.....AAGTTTTTCCAATTTCCATTTCCTCATCT
LUSAD2.SEQ  ..ACAACCATTCAATTCAATATCTCACATTCAAGTTTTTCCAAcTTCCATTTCCTCATCT

LUCDNA.SEQ  GCCTTACCCATAAATCTCGACGGACACCAAAAAACTCAGCCAGCTTGCCCCCAAACAACA 120
LUSAD1.SEQ  GCCTTACCCATAAATCTCGACGGACACCAAAAAACTCAGCCAGCTTGCCCCCAAACAACA
LUSAD2.SEQ  GCCTTACCCATAAATCTC....GACACCAAAAcACTCAGCCAGCTTcgtCCCAAACAAC.

LUCDNA.SEQ  GCGCAGAAAAACCTTCAACAACAATGGCTCTCAAGCTCAACCCAGTCACCACCTTCCCTT 180
LUSAD1.SEQ  GCGCAGAAAAACCTTCAACAACAATGGCTCTCAAGCTCAACCCAGTCACCACCTTCCCTT
LUSAD2.SEQ  ..GCAGAAAAACCTTCAACAACAATGGCTCTCAAGCTCAACCCAGTCACCACCTTCCCTT

LUCDNA.SEQ  CAACACGCTCCCTCAACAACTTCTCCTCCAGATCTCCTCGCACCTTTCTCATGGCTGCTT 240
LUSAD1.SEQ  CAACACGCTCCCTCAACAACTTCTCCTCCAGATCTCCTCGCACCTTTCTCATGGCTGCTT
LUSAD2.SEQ  CgACcCGCTCCCTCAACAACTTCTCCTCCAGATCTCCTCGCACCTTTCTCATGGCTGCTT

LUCDNA.SEQ  CCACTTTCAGTTCCACCTCCACCAAG..............................  300
LUSAD1.SEQ  CCACTTTCAATTCCACCTCCACCAAGTAAG.....CATCTCCTCCTCCTCGGAAT
LUSAD2.SEQ  CCACTTTCAATTCCACtTCCACCAAGTAAGTTCCCGTCACCATCTCCTCtTCCTCGGAAT

LUCDNA.SEQ  ..............................................................  360
LUSAD1.SEQ  CTCCGCCGATTTCTTTTAAGCGATTGATCGTAGATAAATTTGTCGGTTGCTTACCGTTCA
LUSAD2.SEQ  CTCCGCCG.TTTCATTTAAGCGATTGATCGTAGA.AAATcTGTCGGTTGCTTAgCGTTCA

LUCDNA.SEQ  ..............................................................  420
LUSAD1.SEQ  TCAAAATCTGCACGGTTCGTTTCTTCTTCTGCGCCTAGATTGCAT.....TATGTC
LUSAD2.SEQ  TtcAAATCTGCgCGGTTCGTTTCTTtTTCTttcttcAGAcTGCATCATCTGCATTATGTt

LUCDNA.SEQ  ..............................................................  480
LUSAD1.SEQ  ATTGTTCGCTTTCCGATTTGACTGACCGACATAAATCAATTCCTTTGTGTTTCACGATTC
LUSAD2.SEQ  ATTGTTCG.TTTCCGATTTGACTaACCtACATAA.TCAATTCCTTTGTGTTTCACGAgTC

LUCDNA.SEQ  ..............................................................  540
LUSAD1.SEQ  TGGGTTTTGCGCTGTAATTGATTGTCAGTGTTTGCACAGGTTTCCCCTTCTCCTCCTCCG
LUSAD2.SEQ  TGGaTTTTGCGCTGTAATTGATTGTCAGTGTTTGgACAGGTTTCCatTTCTCCaCCTCCG

LUCDNA.SEQ  ..............................................................  600
LUSAD1.SEQ  TCCATCAAATGCATGTTATTACCATTTCAATTTCAGTTTCCTTCTCTGAAATATCCGTCT
LUSAD2.SEQ  TCCATCAAATGCATGTTATTACC.TacCAATTTCAGcgTCtTTCTCTGgAA.....aTtT

LUCDNA.SEQ  ..............................................................  660
LUSAD1.SEQ  CTGGGAAAATAAGTCTCTGTATCTACTATCCTATCAGCTTGTTTAGGAGAGGTTCGATAT
LUSAD2.SEQ  CTG.........TCTCTGTATCTACTATCCTATtAGCTTGTTTgaGAGAGGTTCaATAT

LUCDNA.SEQ  ..............................................................  720
LUSAD1.SEQ  TCGTTTACATAAACCAATTGGCTTACAGTCCTTGAACGTTCTAAATGTTGGTCGCGGTGA
LUSAD2.SEQ  TgGTTTgCATgAACCAAgTGGCTTACAaTCCTTcAACGTTCTAAATGTTGGTCGCaGTaA

LUCDNA.SEQ  ..............................................................  780
LUSAD1.SEQ  TAATAGGTTCTCAAAAGAGGTTTGTCTATGTTGTTTGGCAAAATCTTGTTTCTGTGAATC
LUSAD2.SEQ  cAATAGGTTCTCAAAAGAGGTTTtTCTATGTTGTTTGGCAAAATCTTGTTTCTGTGAATC

LUCDNA.SEQ  ..............................................................  840
LUSAD1.SEQ  ATGTTTAAGGTCCTTGGAAGAATGACTAATGAGCTATGACATGATTACGACGTAGTAGTT
LUSAD2.SEQ  ATGTT.AAGGTCCTgGGAAGAATGAtTAATGAGCTATGACATGATTAaGgCGTAGTAGTT

LUCDNA.SEQ  ..............................................GAGG  900
LUSAD1.SEQ  ATTGAACTGCTGATAATTCAATATAGGGGTAACTTTGTTGATTGTTTGGTCACAGGGAGG
LUSAD2.SEQ  ATTGAACTGCTGATAATTCAATATAGGGGTAACTTTGTTGgTTGTTTGGTgACAGGGAGG
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Figure 1

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LUCDNA.SEQ  CTGA..AGCTAAAGAAGTCACATGGACCACCAAAAGAGGTGCATATGCAAGTGACCCATT  960
LUSAD1.SEQ  CTGAGAAGCTAAAGAAGTCACATGGACCACCAAAAGAGGTGCATATGCAAGTGACCCATT
LUSAD2.SEQ  CTGAGAAGCTAAAGAAGTCACATGGACCACCAAAAGAGGTGCATATGCAAGTGACCCATT

LUCDNA.SEQ  CCATGCCCCCACAGGAAGCTGGGAGATATTTAAGTCTCTGGGAAGGTTGGGGCTGAGGGA 1020
LUSAD1.SEQ  CCATGCCCCCACAG.AAGCTGG.AGATATTTAAGTCTCTGG.AAGGTTGGG.CTGAGG.A
LUSAD2.SEQ  CCATGCCCCCACAG.AAGCTGG.AGATcTTTAAGTCcCTtG.AAGGTTGGG..cagaGGA

LUCDNA.SEQ  TGTTCTTATTTCGCACCTGAAGCCAGTTGAGAAATGCTGGCAGCCACAGGATTTCCTGCC 1080
LUSAD1.SEQ  TGTTCTATTACCGCACCTGAAGCCAGTTGAGAAATGCTGGCAGCCACAGGATTTCCTGCC
LUSAD2.SEQ  cGTTCTgTTgCCGCACCTGAAGCCgGTTGAGAAATGCTGGCAGCCACAaGATTTCCTGCC

LUCDNA.SEQ  CGAACCTGAGTCGGATGGGTTCGAGGAGCAAGTGAAGGAGCTCAGGGCAAGGGCCAAAGA 1140
LUSAD1.SEQ  CGAACCTGAGTCGGATGGGTTCGAGGAGCAAGTGAAGGAGCTCAGGGCAAGGGCCAAAGA
LUSAD2.SEQ  CGAACCcGAGTCGGATGGGTTCGAGGAGCAAGTGAAGGAGCTCAGGGCAAGGGCtAAAGA

LUCDNA.SEQ  ACTGCCCGATGACTATTTTGTTGTGCTGGTTGGGGATATGATCACCGAAGAAGCTCTGCC 1200
LUSAD1.SEQ  ACTGCCCGATGACTATTTTGTTGTGCTGGTTGGGGATATGATCACCGAAGAAGCTCTGCC
LUSAD2.SEQ  ACTcCCCGATGACTATTTTGTTGTGCTGGTTGGGGATATGATCACCGAAGAAGCTCTaCC

LUCDNA.SEQ  GACTTACCAGACAATGCTCAACACCCTTGACGGGGTGAGGGACGAGACTGGAGCCAGCCT 1260
LUSAD1.SEQ  GACTTACCAGACAATGCTCAACACCCTTGACGGGGTGAGGGACGAGACTGGAGCCAGCCT
LUSAD2.SEQ  GACTTACCAGACAATGCTCAACACCCTTGACGGGGTGAGGGACGAGACTGGAGCCAGCCT

LUCDNA.SEQ  TACGCCGTGGGCAATCTGGACAAGGGCGTGGACCGCTGAAGAGAATAGGCACGGTGACCT 1320
LUSAD1.SEQ  TACGCCGTGGGCAATCTGGACAAGGGCGTGGACCGCTGAAGAGAATAGGCACGGTGACCT
LUSAD2.SEQ  TACGCCGTGGGCAATCTGGACAAGGGCGTGGACCGCTGAAGAGAATAGGCACGGTGACCT

LUCDNA.SEQ  TCTCAACAAGTATCTATACCTCTCTGGAAGGGTGGACATGAGGCAAATTGAAAAGACCAT 1380
LUSAD1.SEQ  TCTCAACAAGTATCTATACCTCTCTGGAAGGGTGGACATGAGGCAAATTGAAAAGACCAT
LUSAD2.SEQ  TCTCAACAAGTATCTtTACCTCTCTGGAAGGGTGGACATGAGGCAAATTGAAAAGACCAT

LUCDNA.SEQ  TCAGTATCTCATCGGCTCTGGAATGG..............................  1440
LUSAD1.SEQ  TCAGTATCTCATCGGCTCTGGAATGGTATGTAATCACATACT.........TCATCCTT
LUSAD2.SEQ  TCAGTATCTCATCGGCTCTGGAATGGTATaTAcTCACATCCTATCTGCCCCTTtATCCTT

LUCDNA.SEQ  ..............................................................  1500
LUCAD1.SEQ  TTCTATTAATCTTTGGGTGAACAAAATTCACTACACTGGTAGCAGCTGAAACTTTAGATG
LUCAD2.SEQ  TTCcATTAATCTTTGatTGAACAAAATTCAaTAaACTGGTAGC...TGAAACTTTAGATG

LUCDNA.SEQ  ..............................................................  1560
LUSAD1.SEQ  ATTTTTTTTACTGCCTAGCTTCTATGAAACAAAACCACGTAAGTCAAATAGGGTTGACAA
LUSAD2.SEQ  ATTTgT..TACTGCCTAGCTTCTATGAgA..AAACCACtgAAGTCAAATAGGTTTGACAA

LUCDNA.SEQ  ..............................................................  1620
LUSAD1.SEQ  TGAGTTCAAGTGGCAAAATTTTTCTTATATACCAACTTCGAACCACTTTATATGACATAC
LUSAD2.SEQ  TGgGTTtAAaTGGaAAAAGTT...TcATATACCAtCTTCcAtCtAtTTTATATGACATAC

LUCDNA.SEQ  ..............................................................  1680
LUSAD1.SEQ  CAACTCCTAGTTCGGTTAAAATTCCTC..............CGTCGAAGATATAATACTT
LUSAD2.SEQ  CAACTtCTAcTTtGGagAAAATTCgcCGTGGATAATCATATtaTtGAAGATATAgTACTT

LUCDNA.SEQ  ..............................................................  1740
LUSAD1.SEQ  GG...ATTGGTTAAATGAATTGTGAAAGGATACACGTGATGTGGTCTGGAATTAATTTGT
LUSAD2.SEQ  aGTAGATTGGTTAgATGAAcTGTtAAAcaATACAtGTGATGTcGTgTGcAATTAATTTGT
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Figure 1 (cont.)

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LUCDNA.SEQ  ..........                                                    1800
LUSAD1.SEQ  TTGAATGATCAGTTGGGTTCGGGGCGACAACTGTGAACTGGAACCACCCTAAGTAAATTT
LUSAD2.SEQ  gTaAATGATtAGcTGGGTTCGGGaCGACAAaTGTGAACTGGAA...CCCTA.GTAAA...

LUCDNA.SEQ  ..........                                                    1860
LUSAD1.SEQ  TCTTTCTGTCCTACAAATTTGAGGTTCTCCTTGATCACCTTAGTCCATCTTAGGT....T
LUSAD2.SEQ  ..........CTAtgAATT.GAGGTTgTCCTTcATCACtTTAtTCtgTCcTgGGTCTGTT

LUCDNA.SEQ  ..........                                                    1920
LUSAD1.SEQ  TGCCCGTTAGTAAGATCTGCATTTAGCAGTTTGTCCTGGTATCTGATATCACTAGTATCT
LUSAD2.SEQ  TGCCtGTTtGcAAGATCTGCATgTAGCAGTTTGTCCTGGTATtTGcTAcCAgTgGTATCT

LUCDNA.SEQ  ..........                                                    1980
LUSAD1.SEQ  TTGTTTGATTCCCTAGCATCTCTGAAACCATCGGAC.AAGTAGGTGGTTTAGGACAAATT
LUSAD2.SEQ  TTGTTTGATTCCCTAGCATCTCTGAAAaCATCGGACCAAGTAtcTGGTT.AGGACAAATT

LUCDNA.SEQ  ..........                                                    2040
LUSAD1.SEQ  TGGTTCATTGCGGCATTTTTTGTTTGTATCGCCGTATCATCTGGAAGAAGCAGACAGTTT
LUSAD2.SEQ  TGGTTCATTGCGGCATTTTTTGTTTGTATCGCtGTATCgTCTGGAAGA.GCAGACAGTTT

LUCDNA.SEQ  ..........                                                    2100
LUSAD1.SEQ  TGCAAAGTGGCATCAAGCTCAAGAAAGCAACGGCTAGAAGAAGTTCTACATCTGATGCTT
LUSAD2.SEQ  TGCAAAGTGGCATCAAGCTCAAGAAAGCAACGGCTAGAAGAAGTTCTACATCTGATGCgT

LUCDNA.SEQ  ..........                                                AT  2160
LUSAD1.SEQ  TCCTTTTGTTTCTTTGTGTGCTTTTTGGACTTTGTTCTTTTTTCCTGTAGGATCCAAGAT
LUSAD2.SEQ  TCCTTTTGTTTCTTTGTGTGCTTTTTGGACTTTGTTCTTTTTgCCTGTAGGATCCAAGAT

LUCDNA.SEQ  CCAAAAACAGAAAACAACCCCTACCTCGGTTTCATCTACACCTCATTCCAAGAGAGGGCA  2220
LUSAD1.SEQ  CCAAAAACAGAAAACAACCCCTACCTCGGTTTCATCTACACCTCATTCCAAGAGAGGGCA
LUSAD2.SEQ  CCAAAAACAGAAAACAACCCCTACCTCGGTTTCATCTACACCcCATTCCAAGAGAGGGCA

LUCDNA.SEQ  ACGTTCATCTCCCACGGAAACACAGCCAGACTCGCCAAGGACCATGGGGACATGAAGCTG  2280
LUSAD1.SEQ  ACGTTCATCTCCCACGGAAACACAGCCAGACTCGCCAAGGACCATGGGGACATGAAGCTG
LUSAD2.SEQ  ACGTTCATCTCCCACGGAAAtACgGCCAGACTCGCCAAGGACCAcGGGGACATGAAGCTG

LUCDNA.SEQ  GCGCAGATCTGCGGGATCATCGCAGCAGACGAGAAACGGCACGAAACCGCATACACCAAG  2340
LUSAD1.SEQ  GCGCAGATCTGCGGGATCATCGCAGCAGACGAGAAACGGCACGAAACCGCATACACCAAG
LUSAD2.SEQ  GCGCAGATCTGCGGGATCATCGCAGCAGACGAGAAgCGGCACGAAACaGCATACACCAAG

LUCDNA.SEQ  ATCGTCGAGAAGCTCTTCGAGATCGACCCTGACGGTACAGTGCTGGCACTGGCGGACATG  2400
LUSAD1.SEQ  ATCGTCGAGAAGCTCTTCGAGATCGACCCTGACGGTACAGTGCTGGCACTGGCGGACATG
LUSAD2.SEQ  ATCGTCGAGAAGCTCTTCGAGATCGACCCTGACGGTACAGTGtTGGCtCTGGCGGACATG

LUCDNA.SEQ  ATGAGGAAGAAGATATCGATGCCCGCCCACTTGATGTACGATGGAGAAGACGACAACCTC  2460
LUSAD1.SEQ  ATGAGGAAGAAGATATCGATGCCCGCCCACTTGATGTACGATGGAGAAGACGACAACCTC
LUSAD2.SEQ  ATGAGGAAGAAGATATCGATGCCCGCCCACTTGATGTACGATGGAGAAGACGACAACCTC

LUCDNA.SEQ  TTCGACAATTACTCGTCAGTCGCTCAACGCATCGGGG...ATACTGCCAAGGATTATGCC  2520
LUSAD1.SEQ  TTCGACAATTACTCGTCAGTCGCTCAACGCATCGGGGTGTATACTGCCAAGGATTATGCC
LUSAD2.SEQ  TTCGACAATTACTCGTCgGTCGCTCAACGCATCGGGGTGTATACTGCCAAGGATTATGCt

LUCDNA.SEQ  GATATCCTGGAGTTCCTGGTGGGGAGGTGGAAAGTGGATGCTTTTACGGGGCTTTCCGGG  2580
LUSAD1.SEQ  GATATCCTGGAGTTCCTGGTGGGGAGGTGGAAAGTGGATGCTTTTACGGGGCTTTCCGGG
LUSAD2.SEQ  GATATCCTGGAGTTCCTGGTGGGGAGGTGGAAAGTGGATGCTTTTACGGGaCTTTCCGGG
```

Figure 1 (cont.)

```
LUCDNA.SEQ   GAAGGGAACAAAGCTCAGGATTTTGTCTGCGGGCTTCCTGCGAGGATTCGAAAGTTGGAG 2640
LUSAD1.SEQ   GAAGGGAACAAAGCTCAGGATTTTGTCTGCGGGCTTCCTGCGAGGATTCGAAAGTTGGAG
LUSAD2.SEQ   GAAGGGAACAAAGCTCAGGAgTTTGTCTGtGGGCTTCCaGCGAGGATTCGAAAaTTGGAG

LUCDNA.SEQ   GAGAGGGCTGCGGGGAGGGCAAAGCAAACGTCGAAATCTGTCCCGTTCAGCTGGATCTTC 2700
LUSAD1.SEQ   GAGAGGGCTGCGGGGAGGGCAAAGCAAACGTCGAAATCTGTCCCGTTCAGCTGGATCTTC
LUSAD2.SEQ   GAGAGGGCTGCGGGGAGGGCAAAGCAAACGTCGAAATCTGTCCCaTTCAGCTGGATCTTC

LUCDNA.SEQ   AGCAGAGAATTGGTACTCTAATGGAGTTTGCTTGAGAGT.AGAGTGTGGAATGATTGGAG 2760
LUSAD1.SEQ   AGCAGAGAATTGGTACTCTAATGGAGTTTGCTTGAGAGTTAGAGTGTGGAATGATTGGAG
LUSAD2.SEQ   AGCAGAGAATTGGTACTCTAATGGAGTTTGCccGAGAGTT.GAGTGTGGAATGATTGGAG

LUCDNA.SEQ   TATGATGGCAT                                                 2771
LUSAD1.SEQ   TATGATGGCAT
LUSAD2.SEQ   TATGATGGCAT
```

Figure 1

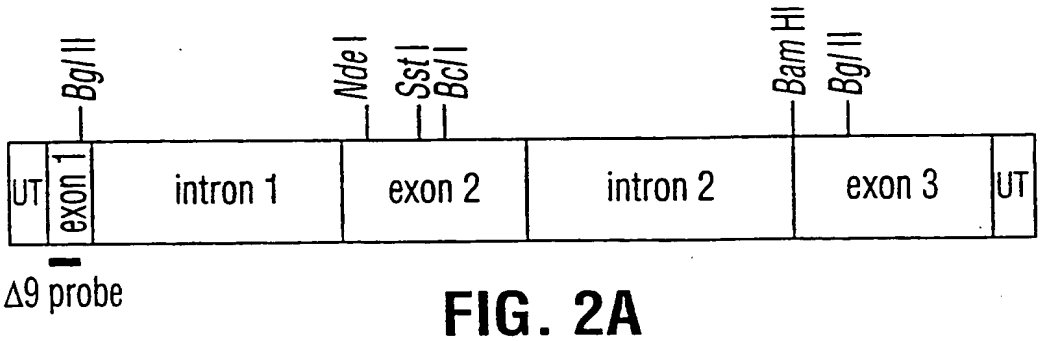

FIG. 2A

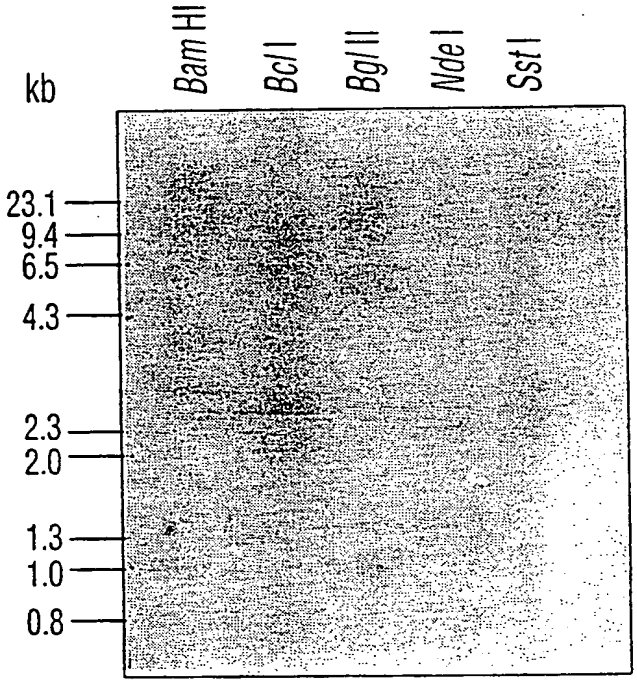

FIG. 2B

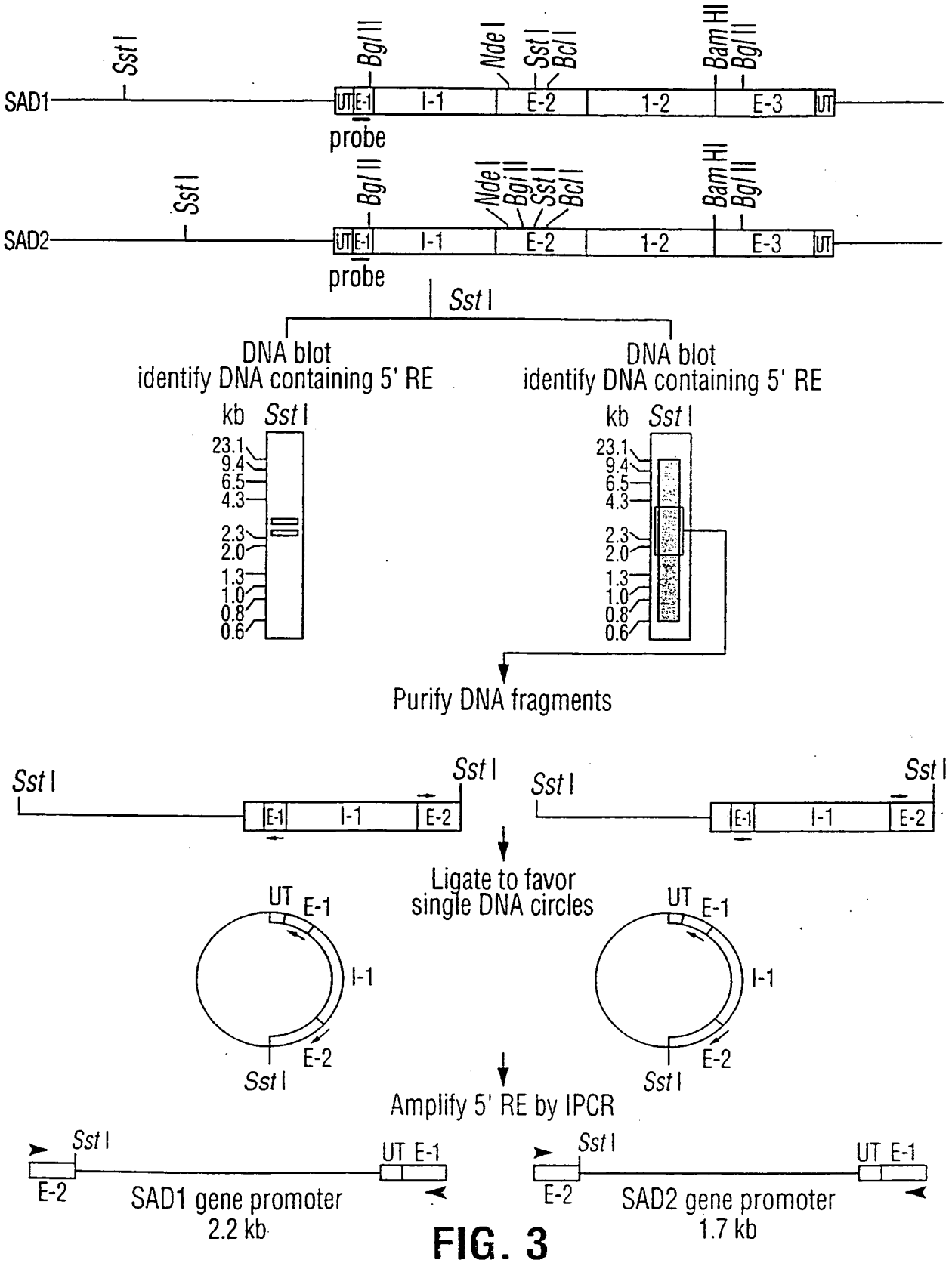

FIG. 3

```
                  SstI
-1692    SAD1: GAGCTCTCAATGTAGTAACACAAAGCCTTCTGTCTTCTTTCTGTAACGTTCAATGCTAGA

-1190    SAD2: -------------------------.....-C-T--T----CA-------G----T----


-1632    SAD1: AC.TTGTCTTCTTATAACTGTTTGTTTGCT.....TCTTCAGCTAATGTTGGAGAAGGAT
-1135    SAD2: --t-------T------------C---CA-gaagc-GA------G---------------

                                                                    XhoI
-1578    SAD1: GGAGCCACGGAGATCCCGGTAAAGCAAAGGATGGATCGAGAGGAGACGGTGGCTCGAGAG
-1075    SAD2: --------------T--T-A---------------A---------------A--------


-1508    SAD1: AACATGGAAGCATTGCACAGAGCCGTCACGTTGGAAGTGCCTCATTCGCAGGCCCCGTCT
-1015    SAD2: T---G------------------T------C-T-C------------AG--TT-TT----


-1458    SAD1: CGGTATGGAACATTTGGTGGTGGTGAGGTTGAAGAAGAGG.AGAAAGATGCCGTAGTTCA
 -955    SAD2: ---------------A----C-----C-------------a----------TA-G-----


-1399    SAD1: TCATCTACTGGGATGGATTGATCC.GCCAGCATGTTCTCCTCCCGAAATCGACCTGTCCC
 -895    SAD2: -------G-----A----------a---G----------------------GG-C-----


-1340    SAD1: TATTGATGACAATGTAACATCAATGTCAATCTCTGCAGATATCTGTTAGGATCAGGTCAT
 -835    SAD2: A---------------------------------------T-T------C-G--------


-1280    SAD1: GATTCTTTTTTGGTTGATTCTTGTGAATGTGTAACATTGATGTAAGCTATTTGTTGTTGT
 -775    SAD2: ------------------------------.....----------------------


-1220    SAD1: AATA..........TCTGATTTTGTTGTTGCTTTGATCAATCAAATAAATCTCGTTCAA
 -728    SAD2: ----tatgcattgat-G----------T-A-----------G-----------------
                    NsiI

-1171    SAD1: CGCGATCATAAGCCTCTTTCATATTCATTTTGACGACTATGT....ATAGTCGTACAAAC
 -688    SAD2: -C-A-C---C--G--------------------------A-atac---A----------


-1115    SAD1: TATTCGGTTAACTAATCTACATCAAGTCGGAATTAGCTAGACATTGTCAAGGAGGAGGAA
 -608    SAD2: --------------------GA--------G---------G-----------.....


-1055    SAD1: AATATCAAGAAAATTGGATGAGGAAATCATACACCCAATTCTGAAGCTGATTCTTCATCT
 -554    SAD2: ......................G----------T----T-----------------


 -995    SAD1: ATGATTTCGAGTTTCGACTTTTTTTGAGTCTCAACTGTGATTTCGAGTTTCGACTTGATT
 -513    SAD2: -.....------------T---------


 -935    SAD1: TGGCTCTTTGATATTCCGAAATTA........AATGCCTCCAAAGTGCTCTCTACTTGCG
 -489    SAD2: --------C------.--------aatgcctc---------------------------


 -861    SAD1: GTTGG.CCTGGTTCANTGGCGAATCATTGAATGACAGAACTAGACAGCTACCAGGTGCAA
 -430    SAD2: -G---a---.....---A-------...--A-A---------
```

Fig. 4

```
-824    SAD1: AAAACATTTGTT........AATGTCTTCTTGCATTAATGTCCATGTTTTCTGCATT.TT
-397    SAD2: -------G----tacacgtcC----TA---------..G-C----------------g-A

                                                                    BglII
-773    SAD1: AATCTTTCCCCAAACACCTAATATATAGCTTCATTGATCCTCCTCTCCACGGTTGCAGAT
-339    SAD2: ----------------.....................................................

-713    SAD1: CTCGTTGCTGATAACACATACATGGCTACAAGACTCTAAAACGGTTCAAAGTGAAATTGT
        SAD2: .....................................................................

-653    SAD1: TTTGGTGGTAGAGTTGTGTGTTTGGTGACTCGAAAGTTCTGGATTCGAATCCAGCATTCC
        SAD2: .....................................................................

-593    SAD1: CCACAAAATAGACACCAACGTAGTGTTTATTTACCGTCTTCTATCTTGTATTGACCGAGA
        SAD2: .....................................................................

                                                              BamHI
-533    SAD1: GTTACGATATACTCCGACAAAAAAAGACATCTTCCACATCATCAAATGGATCCGTAGTTA
        SAD2: .....................................................................

-473    SAD1: GTGCAGTGGCTCGATTAACATAAATGAAAAAAGGAAAAAATTTGCCTGAAATCGATGCTC
        SAD2: .....................................................................

-413    SAD1: AAAACAAGTAGAAATTCATTCAAACATATTTAGACAAACACGATCATTTAGCATCATCAA
-324    SAD2: ..........................G------G--GT----A--C-----C------

-353    SAD1: ATTAATAACAAGAGCAAACAATAAAGCACATAGCAAAACATACAATAGTCGTCTTGCAAT
-287    SAD2: --C-------C--------T-------T-A--.....................................

-293    SAD1: GTCATATGATAATAAGCCAGTGAAACCATGAAGCCCAAGTGAAGTGGTCAAGTGGGAGCT
-256    SAD2: ..................................T--T-----G---.------

              HindIII
-233    SAD1: GAAAGCTT.........CCGAACCCAAGCCCCCGCTACCGGGTTAGGACATACGACACGC
-220    SAD2: --------tcatcggtaT---------C----.....................................

-182    SAD1: GACATGCTACGAAACTTAAAAATCGGTCACGCAGTTA......ATGGAACAAATGAAACG
-188    SAD2: ..-C-------------------G---T----TA---cactcg--A----TG--------

-128    SAD1: CAACGACTATTAAGTGACCATTTTGCAGAAATGAT.ATGAAAAAGTGACCATTTAGACAA
-130    SAD2: ------T-G------A---------------C---a--TG-C------------G--T--

                                                    ________
 -69    SAD1 : ATGAGCAAAGAAAATAC.AGTGGCGAGTGCTGACATAATAAACCGAATGCAGGCGTTACC
 -70    SAD2 : ----C--GG--------a-------------------------------G---------

                      +1
 -10    SAD1 : ATCCAATTTTA
 -10    SAD2 : -----------
```

Fig. 4

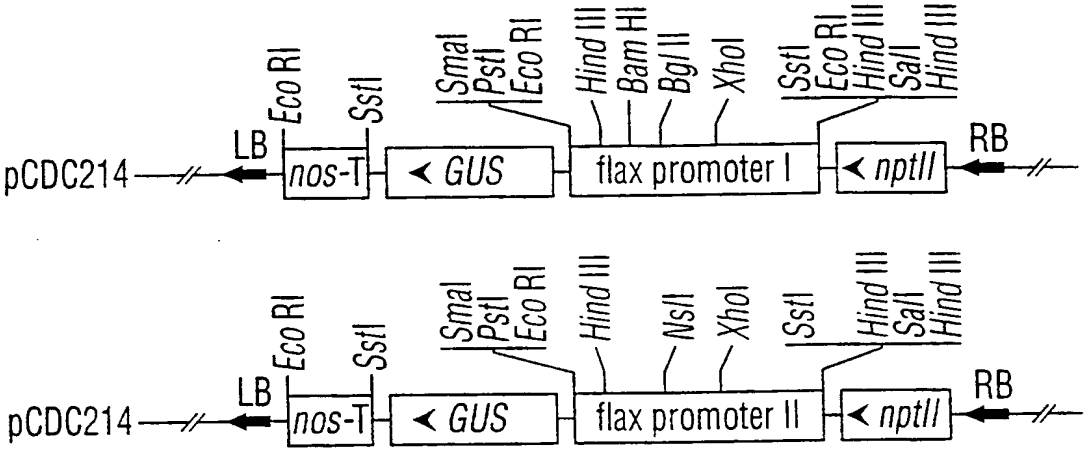

FIG. 5

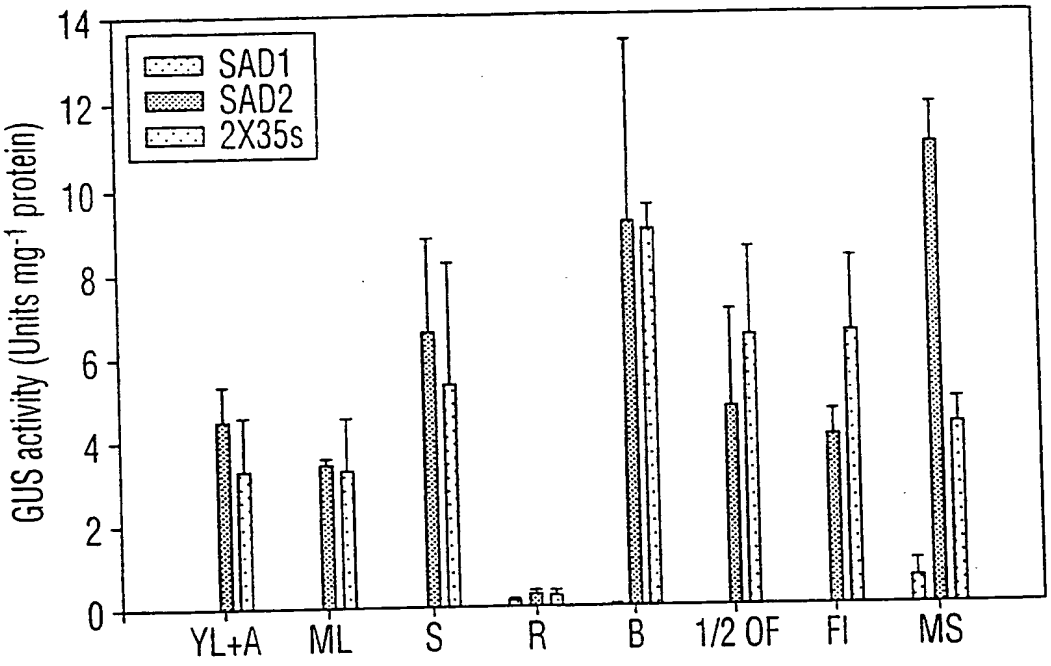

FIG. 6

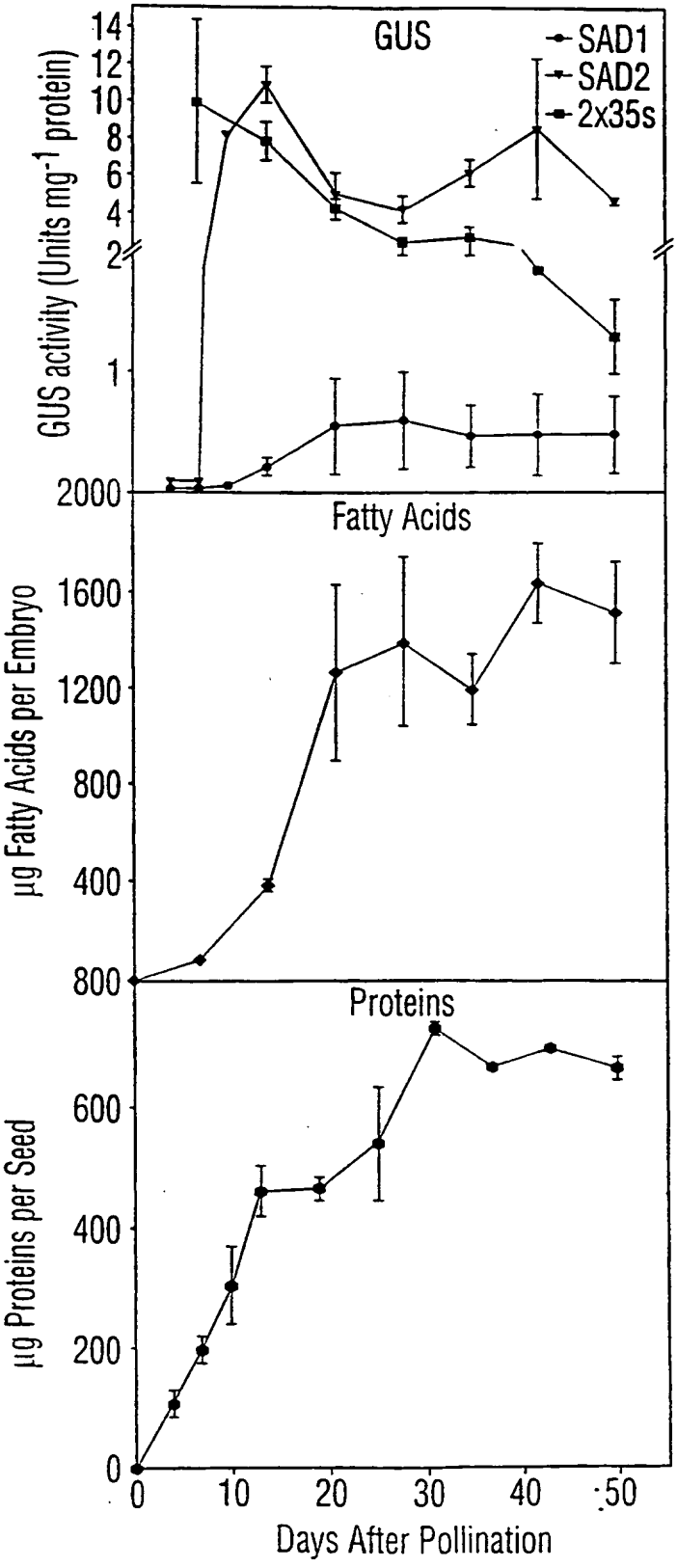

FIG. 7

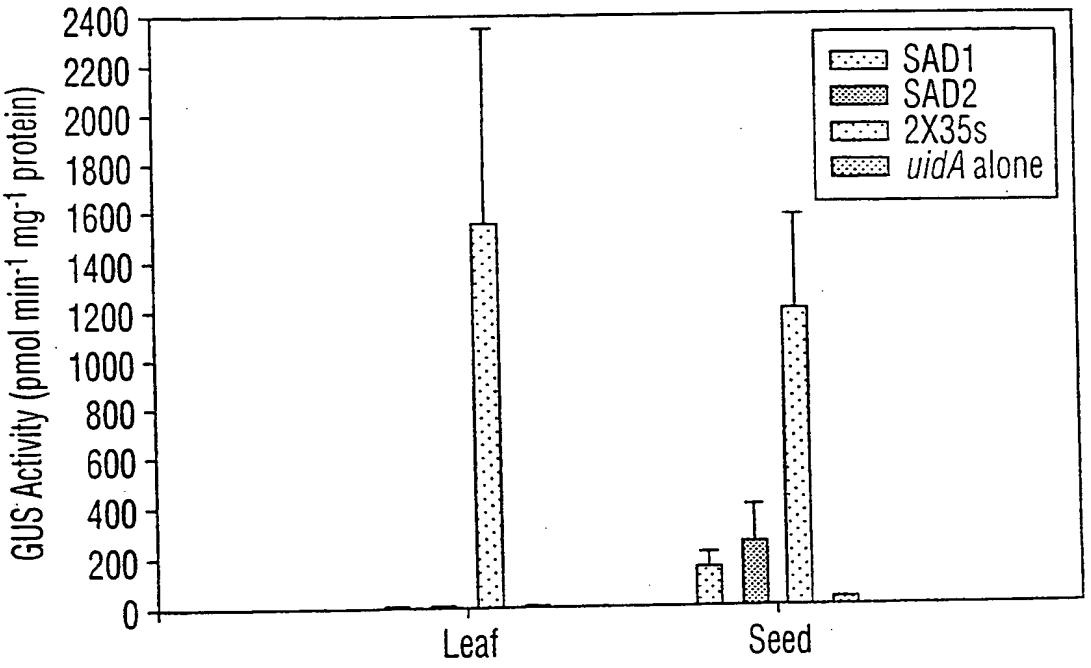

FIG. 8

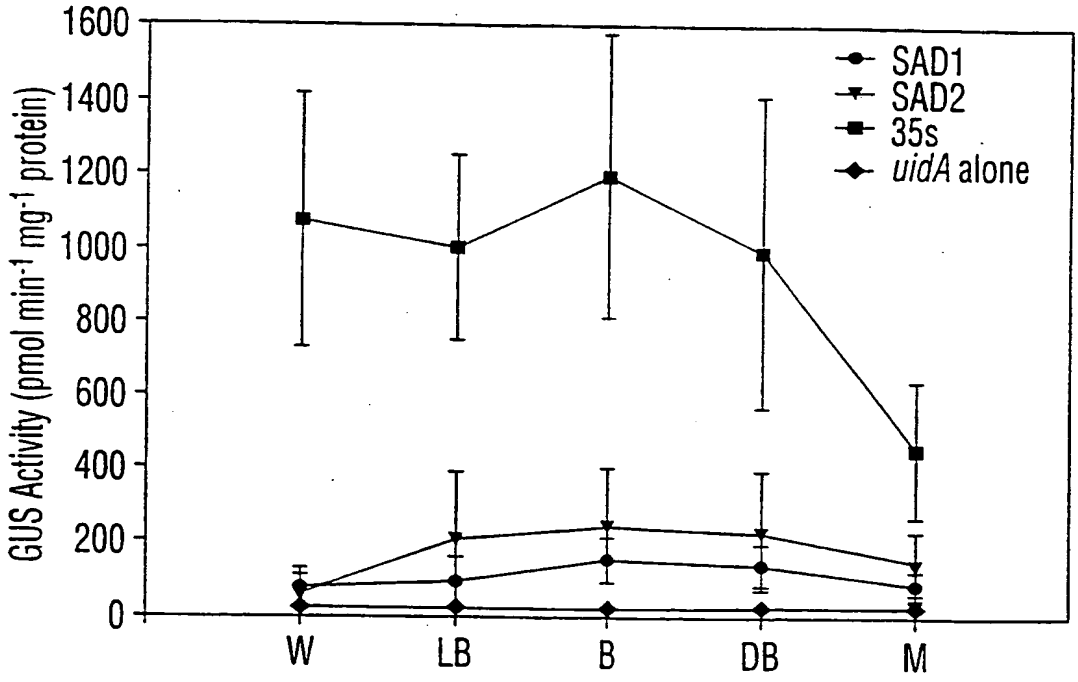

FIG. 9

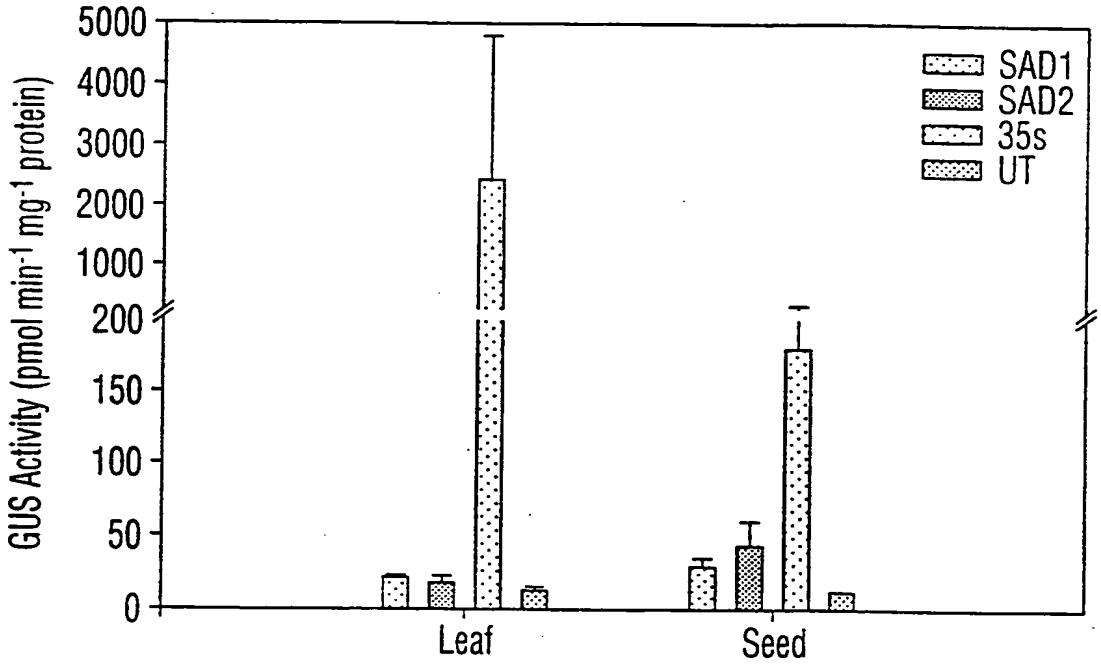

FIG. 10

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 97/00812

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/82 C12N15/53 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

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IPC 6 C12N A01H

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SINGH, S.P., ET AL.: "L.usitatissimum mRNA for stearoyl-(acyl-carrier-protein)-desaturase " EMBL SEQUENCE DATABASE, ACCESSION NO. X70962, 26-FEB-1993, XP002055085	1,7-9,16
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Y	WO 91 13972 A (CALGENE INC) 19 September 1991 see the whole document	10,11,17

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4

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11 February 1998	03/03/1998

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Y A	WO 91 18985 A (DU PONT) 12 December 1991 see page 27, line 10 - page 28, line 15	12,14 10,11,17
A	BIOLOGICAL ABSTRACTS, vol. 101, Philadelphia, PA, US; abstract no. 15165, ROWLAND G G ET AL: "The application of chemical mutagenesis and biotechnology to the modification of linseed (Linum usitatissimum L.)." XP002055122 see abstract & EUPHYTICA 85 (1-3). 1995. 317-321. ISSN: 0014-2336,	1-17
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 97/00812

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A	POLASHOCK, J.J., ET AL.: "Expression of the yeast delta-9 fatty acid desaturase in Nicotiana tabacum" PLANT PHYSIOLOGY, vol. 100, 1992, pages 894-901, XP002055086 see the whole document	10,11,17
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INTERNATIONAL SEARCH REPORT

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(71) Applicant *(for all designated States except US)*: NATIONAL RESEARCH COUNCIL OF CANADA [CA/CA]; 1200 Montreal Road, Ottawa, Ontario K1A 0R6 (CA).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: JAIN, Ravinder, Kumar [CA/CA]; 2413 Irvine Avenue, Saskatoon, Saskatchewan S7J 2A9 (CA). THOMPSON, Roberta, Gail [CA/CA]; 117 Capilano Court, Saskatoon, Saskatchewan S7K 4B9 (CA). ROWLAND, Gordon, Grant [CA/CA]; 213 Lake Crescent, Saskatoon, Saskatchewan S7H 3A1 (CA). McHUGHEN, Alan, Gordon [CA/CA]; 35 Cathedral Bluffs Road, Saskatoon, Saskatchewan S7P 1A1 (CA). MacKENZIE, Samuel, Leonard [CA/CA]; 17 Cambridge Crescent, Saskatoon, Saskatchewan S7H 3P9 (CA). TAYLOR, David, Charles [CA/CA]; 622 Wollaston Bay, Saskatoon, Saskatchewan S7J 4C3 (CA).

(74) Agents: GALE, Edwin, J. et al.; Kirby, Eades, Gale, Baker, Box 3432, Station D, Ottawa, Ontario K1P 6N9 (CA).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

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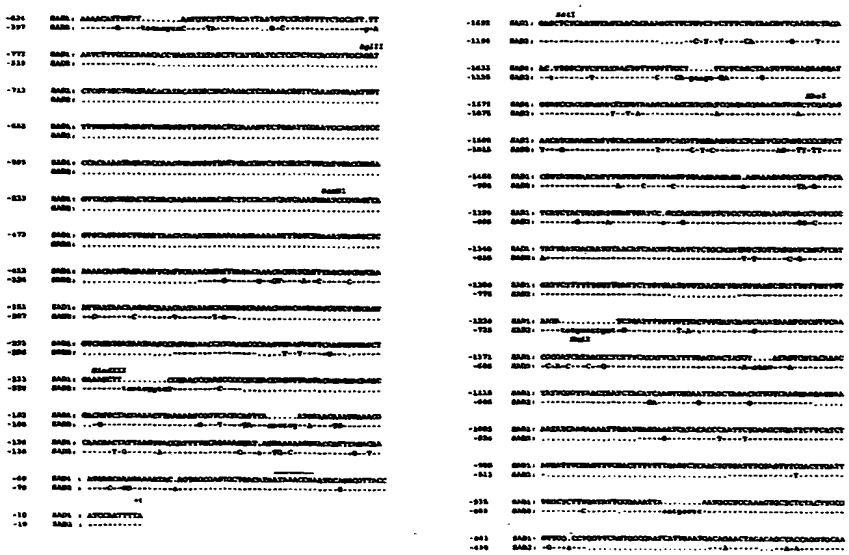
(54) Title: FLAX PROMOTERS FOR MANIPULATING GENE EXPRESSION



(57) Abstract

The invention relates to promoter sequences obtained from flax and useful for modification of flax and other plants for expression of endogenous or foreign genes. The promoters are the SEQ ID NO. 3 and SEQ ID NO. 4 and are obtained from newly illucidated structures of two SAD genes in flax, namely SEQ ID NO. 1 and SEQ ID NO. 2. The promoters have been inserted into cloning plasmids and deposited at the American Type Culture Collection as plasmids pCDC220 and pCDC214 under deposit numbers ATCC 98192 and ATCC 98193, respectively. The promoters may be used in conjunction with genes to modify characteristics of flax and other plants. The invention includes the SAD genes themselves and DNA sequences substantially homologous to SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, and SEQ ID NO. 4, as well as significant parts thereof.

*(Referred to in PCT Gazette No. 38/1998, Section II)

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